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$NiSO_4$ spill inflicts varying mortality between four freshwater mussel species (including protected *Unio crassus* Philipsson, 1788) in a western Finnish river

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Abstract

Freshwater mussels are one of the most threatened taxonomic groups in the world, and many species are on the brink of local or global extinction. Human activities have altered mussel living conditions in a plethora of ways. One of the most destructive human-induced impacts on running waters is the catastrophic spill of harmful substances, which results in massive die-offs. Even though Finland is regarded as the world's top country in terms of environmental regulation quality, riverine systems are not safe. In 2014, River Kokemäenjoki in western Finland experienced the worst $NiSO_4$ spill in the country's history, visibly affecting the mussel community – including protected *Unio crassus* – along the river. Because freshwater mussel toxicology is grossly understudied (particularly in Europe), any pollution –linked die-offs offer valuable opportunities to study the issue in natural environment. Here, we report the mussel investigations from 2014 and a follow-up study conducted in 2017 in order to assess the variation in species sensitivity on nickel pollution. In total, 104 sites were sampled, and over 20 000 mussels were identified and counted. Our results indicate that the most impacted species (i.e. that which experienced the highest spill-induced mortality) was *Anodonta anatina* (62%), followed by *Unio pictorum* (32%), *U. crassus* (24%) and *Unio tumidus* (9%). The underlying reason for the sensitivity of *A. anatina* is not resolved, hence more research is urgently needed. The low mortality among most of the species in 2017 highlights the temporal nature of the pollution impact and the recovery potential of the mussel community. However, the case is more complex with *U. crassus* population, which may be experiencing delayed impacts of the spill. Because nickel is one of the most commonly produced industrial metals in the world (hence the pollution incident risk is high) and River Kokemäenjoki hosts mussel community typical for European rivers, our results may benefit many researchers and stakeholders dealing with riverine environments.

Main finding: The freshwater mussels exhibit differences in $NiSO_4$ pollution tolerance. *Anodonta anatina* is the most sensitive species, followed by *Unio pictorum*, *Unio crassus* and *Unio tumidus*.

Keywords: *Unio crassus*, *Anodonta anatina*, metals, pollution, freshwater mussels, Finland

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1. Introduction

The majority of the world's riverine ecosystems are threatened by human activities (Vörösmarty et al. 2010), thus the state of running waters is a pressing and global concern. Riverine systems are affected by agricultural and urban land use, industrial activities, watercourse alterations and direct species introduction or removal (Malmqvist and Rundle 2002). In addition to the chronic environmental impact inflicted by many of the aforementioned activities, single chemical spills or other isolated incidents may also pose high threats to riverine ecosystems. Even though the impact of a single spill on water quality is usually temporally limited due to water flow, the biota may still be greatly impacted over longer timeframes (Crossman et al. 1973; Soldan et al. 2001; Giger 2009).

Some of the most important invertebrates in rivers are riverine mussels, which contribute to water quality, nutrient cycling and abundance of other benthic organisms (Nobles and Zhang 2011). Unfortunately, many mussel species are endangered due to habitat destruction (e.g. dredging, siltation), declining water quality (e.g. contaminants, eutrophication) and loss of host fish communities (e.g. due to dams) (Williams et al. 1993). According to a recent review, nearly 40% of freshwater bivalve species are near threatened, threatened or extinct, putting them on the top of the list of most threatened taxonomic groups in the world. The situation is most drastic within the order Unionidea: 25 species (2.8%) are confirmed or presumed to be extinct (Lopes-Lima et al. 2018). In order to protect aquatic ecosystems, the European Union is committed, via the Water Framework Directive, to the ambitious goal of ensuring the good quality of surface waters by 2027. Furthermore, two freshwater bivalve species are currently protected within the European Union (*Unio crassus* Philipsson, 1788 and *Margaritifera margaritifera* Linnaeus, 1758; EEC 1992). However, knowledge of the impacts of environmental pollution on adult Unionoid species in European rivers is still scarce, as most of the laboratory studies are conducted on North American species (Havlik and Marking 1987; Beggel et al. 2017). Moreover, laboratory bioassays are conducted in controlled environments, hence the results obtained in these laboratory experiments do not necessarily reflect what would occur in nature. Therefore, any large-scale mussel die-off – especially where the cause and timing are identified – has potential to provide highly important data regarding the sensitivity differences between species, and overall community recovery dynamics. These environmental incidents can provide researchers with a valuable opportunity to study ecotoxicological effects in a natural setting. Such information is crucial in order to protect threatened species, and to offer insight into the sustainable management of rivers.

Nickel is widely utilized industrial metal with great importance on e.g. stainless steel manufacturing (Reck et al. 2008). However, nickel release originating from many anthropogenic sources, e.g. mines (Leppänen et al. 2017) and smelters (Woodfine and Havas 1995) is known to inflict serious damage to aquatic environment. Finland, which ranked highest (out of 75 countries) in the national environmental regulation quality assessment (Esty and Proter 2002), experienced the country's largest industrial nickel (Ni) spill accident in July 2014. The spill entered River Kokemäenjoki, which is inhabited by five freshwater mussel species, including the thick-shelled river mussel, *Unio crassus*. This protected species (EEC 1992) is listed as endangered due to the 50% decline across its range within the past 50 years (Lopes-Lima et al. 2014). The other species inhabiting the river include *Unio tumidus* Philipsson, 1788, *Unio pictorum* Linnaeus, 1758, *Anodonta anatina* Linnaeus, 1758 and *Pseudoanodonta complanata* Rossmässler, 1835.

The main hypothesis in our study is that mussel species exhibit variation in Ni sensitivity resulting clear differences in spill-induced mortality. Since there is a considerable research gap in pollutant sensitivity among European freshwater mussels, the results of this study are very important for authorities dealing with river management and pollution assessment of freshwaters.

2. Materials and methods

2.1 Study site

River Kokemäenjoki is a 120 km long river in western Finland, with an average discharge of 223 $m^3 s^{-1}$. The river is the fifth largest in Finland; the size of the catchment area is 27 046 km^2 (KVVY 2015), which consists mostly of forests (57%), agricultural fields (14%) and water (11%). There is one major city (city of Pori, with ~85 000 inhabitants) and several other smaller towns located by the river (Fig 1). Based on water monitoring data (OIVA Database 2019), the river water (sampled at the city of Pori) is soft (Ca average 8 mg L^{-1} N = 7; Mg 2.4 mg L^{-1} N = 1), nearly neutral (median pH 7.2 N = 385) and classified as eutrophic (total P average 45.7 µg L^{-1} N = 375; total N 1288 µg L^{-1} N = 378). The average electric conductivity is 11.6 mS m^{-1} (N = 282). The water quality has improved considerably during the past decades due to improved industrial waste management practices (KVVY 2015). However, despite these improvements in waste water management, the river is still impacted by point and nonpoint sources of water pollution. In addition, due to the high industrial activity by the river, there is still considerable risk of environmental accidents. One of such incidents was the Norilsk Nickel nickel sulphate ($NiSO_4$) spill in July 2014, which, according to environmental officials, was the largest ever Ni spill in the history of Finland. In total, 66 tonnes of Ni and 94 tonnes of SO_4 were released into the Harjavalta hydropower plant reservoir, located at River Kokemäenjoki. Elevated Ni concentrations were measured for ~20 days after the spill (KVVY 2016; Fig 2). The highest Ni concentration measured after the leak was 8 700 µg L^{-1} (KVVY 2015), and even though the Ni concentrations declined rapidly, high numbers of dead mussels (soft tissue) were reported along the river.

2.2 Sampling

We used mussel mortality data from 2014 and 2017 to assess the impact of the spill on the mussel community along the river and available water monitoring data to assess the spatial and temporal changes in water quality (i.e. the general dynamics of Ni pulse) in the river, downstream of the spill site. The mussel species assemblage and recent (spill-induced) mortality was analyzed from 48 downstream sites and 10 upstream reference sites after the spill (7 July–12 September 2014), and again in 2017 from 39 downstream sites and 7 upstream references sites (between 11 June and 18 August 2017). Transects were placed at approximately 1 km intervals between the spill site and the sea. The site locations were checked on site and moved if sampling was deemed not possible (e.g. dangerously high current velocity). The first transect start was randomly selected (but still placed at an adequate location due to the hydropower dam) within the 1 km distance downstream of the spill site (systematic sampling of mussels; Strayer and Smith 2003). In addition, a total of seven (five in 2014 and two in 2017) qualitative mussel samples were collected where the transect area was not defined.

Due to the depth of River Kokemäenjoki, we used a scuba transect method to collect mussels. The method is regarded as semi-quantitative because visual and tactile searches may miss small and/or buried mussels (Miller and Payne 1988). No excavation samples were conducted, thus the detection probability is unknown. Briefly, a diver proceeded along the pre-installed rope and collected all mussels and shells into a mesh bag. Mussels were identified, classified and counted at the surface and subsequently returned to the original area. The targeted minimum sample size/transect was set to 200 (see e.g. Leppänen 2018). Transect cover area data (available from 53 sites in 2014 and from 44 sites in 2017) was used to calculate mussel density values. To assess mussel mortality, we classified the mussels and shells using a modified post-mortem characteristics-based scale developed by the Michigan Natural Features Inventory (see e.g. Badra 2011), which classifies the mussels as either alive, recently dead, or worn based on the following characteristics:

Alive: shell tightly shut when exposed to air.

Recently dead: soft tissue present or absent; hinge ligament intact; inside shell not chemically altered; most of the periostracum intact; shell has its original strength.

Worn: inner surface covered by periphyton and/or chemically altered; and/or hinge ligament worn; hinge or shell strength distinctly altered.

To assess the spill-induced mortality, we calculated theoretical pre-spill (alive mussels + recently dead mussels) community numbers. To analyze the post-mortem transport of mussel shells (i.e. whether the dead mussels deposited at their site of origin, or transported downstream), we compared the theoretical pre-spill community numbers with the worn shell numbers.

2.3 Statistical methods

We used Spearman's rank correlation (on non-normal count data) to test whether the dead mussels are deposited at their site of origin (i.e. with significance of $P < 0.01$). Mortality hotspots were analyzed using Getis-Ord Gi* analysis (Getis and Ord 1992), using mortality percentages. Briefly, the analysis works by assessing each site value within the context of the neighboring site values. High values are significant only if they are surrounded by high-value neighbor sites. The threshold distance (Euclidean) was set as the average distance at which each sampling site had 8 neighbors (Mitchell 2005). The Getis-Ord Gi* analysis was conducted in ArcMap 10.3.1 (ESRI 2015), and the correlation analysis was conducted with Past 3 statistical software (Hammer et al. 2001).

2.4 Population size calculation

The number of individuals was calculated by interpolating the mussel densities in the systematically placed transects (transects 820–36078; only the NE channel in the Seaward sites was included) to the river segments between them. The average mussel density in the transects at the ends of a segment was multiplied by the area of the segment. The total numbers of mussel species in each segment were added together for the total population size in the impacted area.

3. Results

3.1 Water pollution

The highest measured total Ni concentration in River Kokemäenjoki (8700 µg L^{-1} Ni) was recorded near the spill site on 7 July 2014 in deep (19 m) water (KVVY 2015); the deep water concentrations remained above 250 µg L^{-1} for seven days near the spill site. Background concentrations were reached 20 days after the spill (KVVY 2015). Based on water monitoring data obtained from ELY (2019), the highest surface water concentrations were measured on 7 July near the spill site (2700 µg L^{-1}), and on 8 July near the City of Pori (1700 µg L^{-1} Ni). Surface (1 m) water concentrations remained elevated (values above the National Ni quality threshold index 20 µg L^{-1}; Vuori et al. 2009) for ten days after the spill (Fig 2). The average total Ni concentration (surface water) near the spill site before the accident was 1.9 µg L^{-1} (2003–2013, N = 34) and 4.1 µg L^{-1} (2012–2013, N = 11) at the City of Pori. The average total Ni concentration (surface water) near the spill site in 2017 was 1.7 µg L^{-1}, N=6 and 3.8 µg L^{-1}, N=6 at the City of Pori (OIVA Database 2019).

3.2 Mussel results 2014

In 2014, due to variation in river width, the transect lengths varied from 40 m to 185 m and transect width varied from 0.1 m to 1.5 m. The maximum water depth varied from 2 m to 13 m. The total number of identified mussels (alive mussels and empty shells) from all 58 sites was 11,762. The number of mussels per site varied from 33 (site -17000) to 511 (site -37700), and the average number of mussels per site was 202. Mussel density was highly variable between the 53 sites where the transect width was assessed (average 6.4 mussels per m^2, SD 6.3, range 0–27). The most abundant species (including alive, recently dead, and worn) was *U. pictorum* (3,581; 30%) followed by *U. crassus* (3,199; 27%), *U. tumidus* (3,178; 27%) and *A. anatina* (1,803; 15%). In addition, one specimen of *Pseudoanodonta complanata* was found, but was omitted from further analysis. The clearest difference regarding spatial variation among species was the high abundance of *A. anatina* in the reference sites, whereas in impacted sites, *Unio* species were more abundant. Among the impacted sites, all species showed roughly similar trends: lower densities at Small town sites, and higher densities and variation at City of Pori sites and Seaward sites (Fig 3). Background mortality (i.e. percentage of worn shells) exhibited high variation, and the only common character was the increased percentage of worn shells in the Seaward sites downstream from the City of Pori and in the reservoir located upstream from the spill site (Online Resource 1). Alive and recently dead mussels (theoretical pre-spill community) were positively correlated with worn shells (Spearman's r_s for *A. anatina* = 0.6, $P < 0.01$; Spearman's r_s for *U. crassus* = 0.6, $P < 0.01$; Spearman's r_s for *U. pictorum* = 0.7, $P < 0.01$; Spearman's r_s for *U. tumidus* = 0.7, $P < 0.01$). The recent average mortality (recently dead / alive + recently dead * 100) in sites downstream from the spill site (48 impacted sites) was highest for *A. anatina* (62%; SD 24%), followed by *U. pictorum* (32%; SD 14%), *U. crassus* (24%; SD 22%) and *U. tumidus* (9%; SD 8%) (Fig 4). Pre-spill (alive + recently dead) and post-spill (alive) densities for each species are presented in Table 1 and Fig 3. *A. anatina* exhibited the highest mortality in 39 sites, followed by *U. pictorum,* which exhibited the highest mortality in six sites, and *U. crassus* in three sites. The recent mortality at sites located upstream from the spill site (reference sites) was low (average 0–2.6%). Based on population size calculations, the size of the pre-spill *U. crassus* community was nearly 6.5 million mussels, and the number of spill induced deaths was over one million.

Among the impacted sites, there was considerable variation in recent mortality. *A. anatina* exhibited slightly decreasing mortality from the spill site towards the sea, whereas *U. crassus* and *U. tumidus* showed the opposite trend. Recent mortality for *U. pictorum* did not show any distinct directional changes among the impacted sites. Mortality hotspot analysis detected significant ($P < 0.01$) mortality hotspots for *A. anatina* and *U. crassus* (Fig 3).

3.3 Mussel results 2017 and the post-spill recovery

The 48 follow-up transect samples in 2017 resulted in 11,401 mussels (30 worn and two alive *Pseudoanodonta complanata* were omitted from analysis). Among the 2017 samples, recently dead *A. anatina* were not detected, and the recent mortality was very low for *U. pictorum* (maximum 1.5%). For *U. tumidus,* there was one site where the recent mortality was clearly elevated (site 29803*;* 57% mortality) (Fig 5). *U. crassus* exhibited relatively low recent mortality in multiple sites located in the middle section of the river (maximum mortality at site 9335; 17% mortality) (Fig 5). Based on mean mussel densities (Table 1) *U. tumidus* exhibits most complete recovery in 2017 samples, whereas *A. anatina* and *U. pictorum* occur in nearly identical densities in post-spill 2014 and in 2017 samples. *U. crassus* shows continuous decline in mean density. In reference sites, maximum and average densities for *A. anatina* and *U. tumidus* are considerably higher when compared to 2014 results, whereas for other species, the densities are nearly similar. The abundance of worn mussels in 2017 resembled the abundance of recently dead and worn mussels in 2014 (Online Resource 1 and 2). According to our calculations, the current population size of *U. crassus* is nearly 5 million mussels.

4. Discussion

Our study clearly shows that $NiSO_4$ had varying impact on different mussel species. Because the mussel species identified in River Kokemäenjoki are typical riverine species in Europe (Lopes-Lima et al. 2017), and the density ranges closely agree with commonly reported values for these species (e.g. Weber 2005; Zajac et al. 2016), our results are highly relevant in European mussel ecosystems. Some of the reference sites exhibit low flow conditions (i.e. some sites resemble lake rather than a river) and the high density of *A.anatina* at some of the reference sites is likely attributed to slow flow conditions. The differences in current velocity between reference sites and impacted sites is most probably related to hydropower plant dam located near the spill-site and historical channel manipulation downstream the spill site. The Small towns-sites (starting below the spill site) seem to be less suitable for mussels, likely because of the high flow rates (Layzer and Madison 1994;

Strayer 1999) and higher flow variation due to the hydropower plant (high water release during working hours from peak in electricity demand). At the City of Pori, the flow velocity slows down (Lotsari et al. 2013), which may explain the higher mussel densities at multiple City of Pori and Seaward sites. Since mussels occur in mixed species mussel beds, it can be assumed that all species were equally exposed to the $NiSO_4$ spill along the river.

For example, *U. crassus* were considered to be mostly impacted by agricultural diffuse effects (Lopes-Lima et al., 2014), but recent studies have shown that they may be more tolerant to such factors than previously assumed (Stoeckl and Geist 2016; Inoue et al. 2017). This stresses the importance of understanding the effects of industrial pollution spills investigated in this study of the Kokemäenjoki. Our results resemble those of other high-magnitude riverine pollution incidents of instant deleterious effects on mussel communities (e.g. Crossman et al. 1973; Anderson et al. 1991). In River Kokemäenjoki, all species were impacted along the ~35 km stretch of the river. Interestingly, Getis-Ord Gi* analysis detected mortality hotspots (also visible in curves in Fig 3) for *A. anatina* in sites near the spill, and for *U. crassus* in Seaward areas. This finding suggests differences in the magnitude of $NiSO_4$ impact between species and sites. The mussel tissue analysis (Väisänen 2018; Online Resource 3) conducted in 2014, 2015 and 2017 showed high variation between species, tissues and sampling sites. The clearest spill –related observation was the concentration peak in the 2014 samples retrieved immediately after the spill (*Unio* spp. Ni 4.8 mg kg^{-1} 2.1 SD mg kg^{-1} wet weight, N=7, gills and muscle) and the rapid decline one and half months later (*Unio crassus* Ni 0.39 mg kg^{-1} SD 0.27 mg kg^{-1} wet weight N=12 and *A. anatina* Ni 0.36 mg kg^{-1} SD 0.29 mg kg^{-1} wet weight N=12, gills and muscle). Moreover, the analysis shows that mussel gills exhibit higher Ni concentrations when compared to muscles. There is an overall higher Ni concentration downstream from the spill site also in 2015 and 2017, but this is difficult to relate to the spill because the Ni concentration in river water was 2 times higher at the City of Pori when compared to spill site before the accident. Typically, the heavy metals concentrations vary among species, tissues and sampling sites (*A. anatina, U. pictorum*; Gundacker 2000; *A. anatina, U. tumidus*; Rzymski et al. 2014). As such, the metals accumulation is not considered an ideal method for assessing the toxic impact (Zuykov et al. 2013). Typically, heavy metals uptake is first very rapid and then levels off. Similarly, once the environment is uncontaminated, the elimination of heavy metals is very rapid at first, followed by slower elimination (Thorsen et al. 2006), which explains the reported metals concentration peaks immediately after the spill and lower metals concentrations a few months later.

The mussel species did not exhibit any clear preferences in terms of habitats (patchy distribution and multi-species mussel beds is commonly reported; Watters 2006; Allen et al. 2013; Lopes-Lima et al. 2015), which could have been used to assess the reasons for differences in mortality. In addition, the filtration rates for *A. anatina*, *U. crassus*, *U. tumidus* and *U. pictorum* are nearly identical (Kryger and Riisgård 1988) and *A. anatina, U. tumidus* and *U. pictorum* exhibit similar activity levels and grain size preferences in pedal feeding (benthic material is taken up by the mussel foot and transported into the digestive system) (Brendelberger and

Klauke 2009). Subsequently, habitat preferences or variation in feeding/filtration activity do not seem to explain the mortality differences.

According to a recent review, the most important toxicity mechanisms of Ni on aquatic organisms are disruption of homeostasis of Ca^{2+}, Mg^{2+} and/or $Fe^{2+/3+}$, oxidative damage and an allergic –type response at respiratory tissues (Brix et al. 2016). In case of River Kokemäenjoki, multiple of aforementioned mechanisms may have simultaneously contributed to the observed negative impacts. There is a lack of toxicological thresholds for European *Unio* species regarding Ni (Naimo 1995; Farris and Van Hassel 2006), making it difficult to assess the tolerance variation between species. However, measured Ni concentrations exceed the acute toxicological thresholds for juvenile North American *Unio* species (173–676 µg L^{-1} LC50 for four Unioidea and one Margaritiferidae species in a 96h test; Wang et al. 2017). For juvenile *Utterbackia imbecilis*, Ni LC50 was 240 µg L^{-1} in a 48h test (Keller and Zam 1991), and for juvenile *Hamiota perovalis* and *Villosa nebulosa*, the Ni EC50 values for a 96h test were 313 µg L^{-1} and 510 µg L^{-1}, respectively (Gibson et al. 2018). Thus, despite the relatively short period of extremely high Ni exposure, the spill most likely had direct toxic impacts on mussels along the river. Mussels are known to detect harmful substances in the water and to escape short-term contamination by closing their shell (e.g. Naimo et al. 1992; Pynnönen and Huebner 1995), although this pollution avoidance mechanism is only temporary because of their metabolic needs (Cope et al. 2008). Douda (2010) reported that *A. anatina* is more sensitive than *U. crassus* to nitrate pollution, thus the variation in species-level sensitivity may explain the differences in mortality. Further, there is interesting species-level variation in the mussels ability to detect harmful elements; *A. anatina* did not exhibit shell closure during Hg exposure and was thus eliminated, whereas *Corbicula fluminea* did exhibit shell closure and was not harmed, even at the highest Hg concentration (Oliveira et al. 2015). Pynnönen (1990) reported the highest Ca^{2+} hemolymph concentrations for *U. tumidus* and *U. pictorum,* while it was lowest for *A. anatina*. One of the most important toxic mechanisms of Ni in freshwater animals is disruption of Ca^{2+} homeostasis (Brix et al. 2016), and Ca^{2+} is known to compete with metal ions by a variety of ways, such as blocking the entry of metals inside the cells (e.g. de Paiva Magalhaes et al. 2015). Thus, species-level differences in mussel biology may also explain the variation in Ni tolerance.

According to the OIVA Database (2019), River Kokemäenjoki surface water temperature in Harjavalta town was around 25°C in July–August 2014. Since this temperature was considerably high (average July–August temperature has been 19.2°C between 1961–2018, N 60; OIVA Database 2019), the mussels may have been negatively affected by elevated temperatures (Gagnon et al. 2014; Golladay et al. 2004; Haag and Warren 2008; Falfushynska et al. 2014). In River Rhine, *A. anatina, U. tumidus* and *U. pictorum* are restricted to sites where the water temperature does not exceed 24°C (*A. anatina, U. tumidus*) or 28°C (*U. pictorum*) (Verbrugge et al. 2012). Thus, even though it does not explain the mortality differences between species, the abnormally high temperature can be considered as an additional stressor during the spill. In addition to direct temperature stress, the high water temperature during the spill may have shortened the period of valve closure. Metabolic needs (oxygen demand) increase in warm water, while oxygen solubility in warm water decreases (Galbraith et al 2012). Thus, the high temperature during the $NiSO_4$ spill may have intensified the exposure to pollution because of the resulting increased filtration volumes (Beggle et al. 2017) and shortened period of valve closure. Further, according to Brix et al. (2016), one of the toxic effects of Ni in aquatic biota is the impaired respiration, which may have had negative synergic effect with high temperature.

The differences in species recovery are difficult to untangle, but the differences could be related to higher spill-induced juvenile mortality among *A. anatina*, *U. crassus* and *U. pictorum* when compared to *U. tumidus*. Indeed, if the small individuals were lost from the population in 2014 spill, the population recovery would have been delayed. In addition, the decline of *U. crassus* density and relatively high recent mortality (when compared to other species) in 2017 below the spill site is particularly interesting. This observation means that the *U. crassus* population has been on the decline since 2014. The proportion of worn *U.crassus* (which indicates the mortality on longer term; Online resource 1) was not higher when compared to other species in 2014 which suggests that the *U. crassus* mortality was not elevated before the spill. Species specific "semi-chronic" mortality, which recurs over multiple years and phenomenon of delayed mortality, has been observed in many mussel die-off cases (Richard 2018). Higher mortality of *U. crassus* in 2017 seems not to be connected with tissue Ni concentrations (Online resource 3), because the highest concentrations in 2017 were measured in *A. anatina* samples.

Population size (or number of recently dead mussels) estimation is extremely difficult due to the lack of data regarding detection probability. Another issue is the spatial heterogeneity typical for mussels, which is reflected here as high variation in mussel density between sites. This is highlighted in reference sites, where the number of transects is low and the mussel density differences between 2014 and 2017 may be effected by transect placement in high-density sites. Nevertheless, crude numbers can be retrieved by simple extrapolation of mussel numbers. Despite the high uncertainty regarding these numbers, it is likely that as many as 16 million mussels were living in the impacted river section before the spill, and the magnitude of die-off could have been around 4 million mussels (1 million of *U. crassus*).

5. Conclusion

In conclusion, although our results clearly indicate that *A. anatina* was the most sensitive species to the $NiSO_4$ pollution plume (followed by *U. pictorum, U. crassus* and *U. tumidus*), the underlying mechanism of this increased sensitivity remains unsolved. It seems that *A. anatina* holds some potential for early warning species in northern boreal riverine systems. However, more research is clearly needed. Most urgently, the basic toxicological studies should be conducted for adult European unionid species. In addition, the species variation in their ability for prolonged shell shutdown is an important issue that should be clarified with European species. The role of elevated temperatures should also be studied, since the ability to keep the shell shut is probably affected by temperature due to elevated metabolism and lower oxygen content in warm water. This is especially relevant in light of recent and future climate change. Moreover, the phenomenon of delayed mortality should be studied in order to understand the temporal aspect (and actual total impacts) of pollution incidents.

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Figure captions

Fig. 1 a: Location of River Kokemäenjoki; b: River Kokemäenjoki catchment; c: Study area. Sampling sites are labeled according to their distance (m) from the Harjavalta hydropower plant reservoir dam. The spill site is indicated by the black arrow. The river flow direction is from SE to NW towards the Bothnian Sea. The red dot represents the approximate location of central Pori. Ulvila, Nakkila, Harjavalta and Kokemäki are small towns located by the river. Seaward, City of Pori and Small towns describe the general character of land area to the adjacent river section. Coordinates are in WGS84 system.

Fig. 2 Post-spill Ni concentration dynamics in River Kokemäenjoki. The values were obtained from ELY (2019). The arrow indicates river flow direction (N = 28). Filled circles represent sampling sites. Interpolation maps are created using spline with barrier -method in ArcMap 10.3.1 program (ESRI 2015). River width has been edited for ease of interpretation.

Fig. 3 Post-spill mussel mortality. White bars indicate pre-spill density (recently dead + alive mussels) and black bars indicate the post-spill density (alive mussels). Bottom x-axis is for density data. The line indicates the mortality percentage, upper x-axis is for percentage data. Gray areas represent statistically significant mortality hotspots ($P < 0.01$).

Fig. 4 Mussel post-spill mortality % at impacted sites. The box indicates 25–75 percent quartiles, whiskers denote maximum and minimum values and horizontal line indicates median value.

Fig. 5 2017 mussel mortality. White bars indicate recent density (recently dead + alive mussels) and black bars indicate the 2017 density (alive mussels). Bottom x-axis is for density data. The line indicates the mortality percentage, upper x-axis is for percentage data. Note the scale is different for *U. pictorum* percentage data (x-axis).